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TRANSMITTAL FORM

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Application Number	08/869,386
Filing Date	06/05/97
First Named Inventor	Sastry et al.
Art Unit	1648
Examiner Name	Le, Emily M.
Attorney Docket Number	UTXC:538US

Total Number of Pages in This Submission

ENCLOSURES *(Check all that apply)*

- ☐ Fee Transmittal Form
  - ☐ Fee Attached
- ☐ Amendment/Reply
  - ☐ After Final
  - ☐ Affidavits/declaration(s)
- ☐ Extension of Time Request
- ☐ Express Abandonment Request
- ☐ Information Disclosure Statement
- ☐ Certified Copy of Priority Document(s)
- ☐ Reply to Missing Parts/ Incomplete Application
  - ☐ Reply to Missing Parts under 37 CFR 1.52 or 1.53
- ☐ Drawing(s) (Figs. 1 and 2 on 2 sheets)
- ☐ Licensing-related Papers
- ☐ Petition
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David L. Parker

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
SASTRY *et al.*

Serial No.: 08/869,386

Filed: 06/05/97

For: COMPOSITIONS AND METHODS FOR ELICITING AN IMMUNE RESPONSE

Group Art Unit: 1648

Examiner: Le, Emily M.

Atty. Dkt. No.: UTXC:538

SUPPLEMENTAL BRIEF ON APPEAL

I. Real Party in Interest

The Real Party in Interest is the assignee, Board of Regents of the University of Texas System.

II. Related Appeals and Interferences

There are no related appeals or interferences.

III. Status of Claims

Claims 1-28, 36-40, 46 and 48 are canceled. Claims 29-35, 41-45, 47 and 49 are pending and subject to rejection.

IV. Status of Amendments

An amendment canceling claims 36-40 is filed concurrently herewith to reduce issues for appeal and will presumably be entered.

V. Summary of Claimed Subject Matter

The invention of claim 29 is concerned with method for directly inhibiting HIV entry into a cell comprising the step of contacting said cell with a composition comprising a peptide of 8 to 24 residues comprising the sequence RAFVTIGK (SEQ ID NO:5), wherein said cell is in a human subject. Specification, page 15, lines 23-28; page 17, lines 24-34.

The invention of claim 30 recites that the peptide is 8 residues in length. Specification, page 66, lines 12-33, particularly line 25.

The invention of claims 31-35, concern peptides of 15 residues in length (claim 31), those having the sequence RIQRGPGRAFVTIGK (SEQ ID NO:1) (claim 32), peptides of 24 amino acids in length (claim 33), those having the sequence NNTRKSIRIQRGPGRAFVTIGKIG (SEQ

ID NO:3) (claim 34), and peptides in the form of a multimer (claim 35). Specification, page 66, lines 12-33.

The invention of claims 41–45, 47 are directed to inclusion of the peptides in a pharmaceutically acceptable aqueous medium (claim 41), administration at a dosage range of between about 10 micrograms to about 500 milligrams (claim 42), a dosage range is about 50 micrograms to about 1 milligram (claim 43), a dosage of about 100 micrograms (claim 44), contacting said cell with said composition a second time (claim 45), and administration by injection (claim 47). These are described in the same sections mentioned above, as well as on pages 36 – 40.

Lastly, claim 49 is concerned with a method for directly inhibiting HIV entry into a cell *in vitro* comprising the step of contacting said cell with a composition comprising a peptide of 8 to 24 residues comprising the sequence RAFVTIGK (SEQ ID NO:5). See Example 8 of the specification.

VI. Grounds of Rejection to Be Reviewed on Appeal

The grounds of rejection to be reviewed on appeal include:

Whether the meaning of the term "directly inhibiting" is indefinite under 35 U.S.C. §112, second paragraph.

The rejection of claim 49 as allegedly anticipated by Koito *et al.*

The rejection of the remaining claims as obvious over Haynes et al., US 5,019,387, in view of Koito *et al.*

VII. Argument

A. Miscellaneous Issues

In the subject Action, a number of issues not pertinent to the present appeal have been raised by the Action. Appellants are merely stating here how these issues will be dealt with should Appellants prevail in the present appeal:

1. Priority

Appellants agree that the present claims are enabled as of and entitled to the September 16, 1992 filing date. Appellants will amend the continuing data accordingly following appeal.

2. Section 112, Second Paragraph Issues

In the Action, the Examiner requested clarification regarding the identity of SEQ ID NO:3. In this regard it is stated that the Examiner's identification of the sequence on page 4, line 5, of the subject Action is correct. Regarding the Examiner's request for a full and complete sequence listings, such have already been supplied in Applicants' submissions of September 29, 1997.

With regard to claims 43-44, the dependencies of these claims will be changed to address the antecedent basis issue such that these claims will depend from claim 42.

Regarding the issue with respect to claims 31-34, the dependencies of claims 32 and 34 will be changed to address the Examiner's concerns.

Regarding claims 36-40, these claims have been canceled. The issue with respect to claim 43 will be moot upon the change of dependency of this claim.

B. Rejection of Claims Under 35 U.S.C. §112, Second Paragraph

The Action rejects claims 29-45 and 47 under 35 U.S.C. 112, second paragraph, taking the position that it is "unclear the activity that is intended by the limitation 'directly inhibiting HIV entry into the cell'."

In response, it is submitted that "directly inhibiting" or "inhibiting" the "entry of HIV into a cell" is clearly explained in the specification and exemplified by the assay described beginning at page 66, line 20, wherein peptides such as the R15K peptide are tested for their ability to directly inhibit the entry of HIV across a cell having an intact cell membrane. This definition is indeed consistent with the excerpt referred to by the Examiner at the top of page 5 of the subject Action.

Furthermore, "directly inhibiting" or "inhibiting" the "entry of HIV into a cell" is distinguished in the present specification from inhibition of syncytia formation, which is described at page 69, lines 8-23, of the specification. As noted, the test for inhibition of syncytia formation involves ***pre***-infecting target cells with HIV ***followed*** by treating them with the peptides and observing whether the peptides reduce the formation of syncytia. Furthermore, at page 37, lines 15-29, particularly lines 28-29, it is clearly denoted that inhibition of HIV infection is intended to be distinct from inhibition of syncytia formation (noting that the reference of Koito *et al.*, which concerned inhibition of syncytia formation, did not test "these peptide [] for their capacity to inhibit HIV infection of cells.").

It is submitted in light of the foregoing that the claims are acceptable under 35 U.S.C. 112, second paragraph.

C. Anticipation of Claim 49

The Action next rejects claim 49 as anticipated by Koito *et al.* reference.

In response, Appellants are entirely unsure as to how the Examiner considers Koito *et al.* to be relevant to anticipate the subject matter of claim 49. At best, Koito *et al.* merely indicates that an R15K peptide inhibits syncytia formation. Nowhere does Koito *et al.* teach inhibition of HIV entry into a cell. Although the Action is short on explanation, it is presumed that the Examiner is taking the position that inhibition of syncytia formation necessarily results in a reduction in viral entry. Yet, there is no evidence of record to demonstrate this to be the case, and there is no demonstration anywhere in Koito *et al.* that virus entry is in any way inhibited. Thus, no *prima facie* rejection has been made.

D. Obviousness Rejection of Claims

Lastly, the Action rejects the remaining claims over the combination of Haynes *et al.* with Koito *et al.*

With respect to Haynes *et al.*, the Action concedes that:

1) "[T]he cell used in the method of Haynes *et al.* is from a primate. Haynes *et al.* does not specify the primate as a human subject." (Action, page 9);

This is particularly relevant in that the claims subject to rejection are all directed to the treatment of human subjects. The Action fails to provide any explanation how studies involved primates are relevant to the present human therapeutic claims.

2) "[T]he mechanism of action that Haynes *et al.* describes for the composition is not the same as that observed by Applicant. Haynes does not describe the mechanism of action as direct inhibition of HIV entry into cells."

This is also a critical shortcoming in that present claims are specifically directed to a specific mechanism which is admittedly not taught or suggested by Haynes *et al.* Inherency is not an issue in that Haynes *et al.* is concerned with vaccination to induce an immune response – presumably by intradermal or some similar administration – whereas the present invention involves introduction into the bloodstream. Furthermore, Haynes *et al.* concerns producing immunity by inducing an antibody response but says nothing about the present invention which is directed to inhibiting HIV uptake into cells. Furthermore, as mentioned below, the peptides of Haynes *et al.* relied upon by the Examiner are not the same as those set forth in the pending claims, and thus the doctrine of inherency is inapplicable.

The fact remains, Haynes *et al.* admittedly fails to teach or suggest that the presently claimed peptides can inhibit HIV entry into cells.

3) Haynes *et al.* does not specifically disclose the claimed sequence (Action, page 11)

This observation is particularly damning of the Action's rejection. The Action attempts to argue that the peptides of Haynes et al. are somehow interchangeable with those of the present claims, but there is no basis recited for such a proposition. Indeed, the principle peptide structures relied upon by Haynes *et al.* can be found in Table I at col. 4 – yet these peptides are totally unrelated to the present R8K peptides! The peptides in Table II do contain an R8K sequence, but the addition of these sequences are merely indicated as "enhancing" the ability to raise type-specific antibodies, as opposed to themselves being able or responsible for achieving an antibody response. Col. 6, lines 11-19.

With respect to the Action's reliance on Koito *et al.* to complete the rejection, Appellants direct the Board to the discussion above with respect to the distinctions between syncytia formation inhibition and inhibition of HIV entry. The foregoing is most notably seen when one looks at the

concentrations of peptide required by Koito *et al.* to demonstrate inhibition of syncytia formation. The relevant Koito et al. peptide cited by the Examiner was "NNT24". As shown in Table 1, this peptide was only active in inhibiting syncytia formation at concentrations of 100 μM and above. However, the present Applicants have demonstrated that the presently claimed peptides are active at exceedingly lower concentrations – on the order of 0.4 to 0.78 μM – more than two orders of magnitude lower concentration!

This finding is indeed of substantial significance. Koito *et al.* states at the top of page 617, col. 1, that "[t]he high concentration (0.03 – 1 mM) needed to inhibit syncytia formation described here suggests that the [clinical] usage might not be practical." In other words, compositions that are only active at high concentrations such as 30 to 1000 μM (*ie.*, 0.03 – 1 mM) are simply not good candidates for pharmaceuticals. Thus, one of skill would not be motivated from Koito et al. to use the peptides it discloses as a pharmaceutical – they are simply too inactive for the purpose of syncytia formation. However, Appellants discovery that such peptides are active at more than a two-log fold lower concentration, by an unrelated mechanism, now demonstrates their clinical promise.

Lastly, Appellants will close by making of record the PTO's position in this appeal that the utility claimed by Appellants here would not have been believed by one of skill in the art as of the filing date:

> The specification provides no probative evidence to support the claimed treatment which would protect humans against HIV infection. The obstacles to treatment development and therapeutic approaches with regard to retroviruses associated with AIDS in humans are well documented in the literature. These obstacles include: 1) the extensive genomic diversity associated with the HIV retrovirus, particularly with respect to the gene encoding the envelope protein, 2) the fact that the modes of viral transmission include virus-infected mononuclear cells, which pass the infecting virus to other cells in a covert form, as well as via free virus transmission, 3) existence of a latent form of the virus, 4) the ability of the retrovirus

to "hide" in the central nervous system where blood cells and neutralizing agents carried by the blood cannot reach the retrovirus, due to the blood-brain barrier and 5) the complexity and variation of the elaboration of the disease. The existence of these obstacles establish that the contemporary knowledge in the art would prevent one of ordinary skill in the art from accepting any vaccine or any immunization treatment or any therapeutic regimen on its face. In order to enable claims to drugs and their uses, either *in vivo* or *in vitro* data, or a combination of these can be used. However, the data must be such as to convince one of ordinary skill in the art that the claims are sufficiently enabled. When the claims are directed to humans adequate animal data would be acceptable in those instances wherein one of ordinary skill in the art would accept the correlation to humans. Thus in order to rely on animal data there must exist an art-recognized animal model for testing purposes. See In re Hartop, 311, F,2d 249, 135 USPQ 419 (CCPA 1962).

Yarchoan *et al.* (J. Enz. Inh., 1992) state that while a number of agents have been found to block HIV binding to the target ell *in vitro*, these agents have generally not shown clear-cut evidence of clinical activity (abstract). Moreover, Gait *et al.* (TIBTECH 1995) discuss the problems associated with protein therapies for HIV and state that they suffer from problems of short serum half-life, poor bioavailability, and rapid clearance. Gait *et al.* also teach that as these problems were overcome, other problems emerged such as sequestration of the drug by serum proteins, drug resistance, and uneven distribution through the body, and that since these types of problems are unpredictable, it remains necessary to take into account the pharmacological parameters p. 437).

Factors to be considered in determining whether a disclosure would require undue experimentation have been summarized in Ex parte Forman, 230 USPQ 546 (BPAI 1986) and reiterated by the Court of Appeals in In re Wands, 8 USPQ 2d 1400 at 1404 (CAFC 1988). In the instant specification, it is determined that: 1) there are no working examples which suggest the desired results of inhibiting HIV infection *in vivo*, 2) the nature of the invention involved the complex and incompletely understood area of immunity to HIV, 3) the state of the prior art shows that prior treatment methods have been largely ineffective for the intended purpose, 4) the relative skill of those in the art is commonly recognized as quite high (post-doctoral level), and 5) the lack of predictability in the field to which the invention pertains is recognized in the art as evidenced by prior failures. In view of all of the above, it is determined that the specification is not commensurate ins cope with the claimed invention.

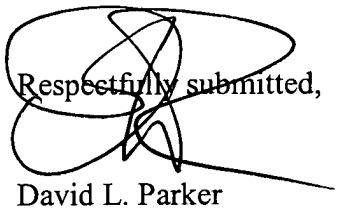
Examiner's Answer dated 4/25/00, paper 21.

We submit that the Action must provide an explanation as to why these observations do not in themselves provide strong evidence of the non-obviousness of the present invention.

In light of the foregoing, the Board is requested to overturn the obviousness rejection.

Conclusion

Appellants believe that the foregoing remarks fully and appropriately respond to all of the Examiner's rejections. The Board is therefore requested to rule in Appellants favor and overturn the rejections.



Respectfully submitted,

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Date: January 13, 2006

VIII. Claims Appendix 1

1. – 28. (Canceled)

29. (Previously presented) A method for directly inhibiting HIV entry into a cell comprising the step of contacting said cell with a composition comprising a peptide of 8 to 24 residues comprising the sequence RAFVTIGK (SEQ ID NO:5), wherein said cell is in a human subject.

30. (Previously presented) The method of claim 29, wherein said peptide is 8 residues in length.

31. (Previously presented) The method of claim 29, wherein said peptide is 15 residues in length.

32. (Previously presented) The method of claim 31, wherein said peptide comprises the sequence RIQRGPGRAFVTIGK (SEQ ID NO:1).

33. (Previously presented) The method of claim 29, wherein said peptide is 24 amino acids in length.

34. (Previously presented) The method of claim 33, wherein said peptide comprises the sequence NNTRKSIRIQRGPGRAFVTIGKIG (SEQ ID NO:3).

35. (Previously presented) The method of claim 29, wherein said peptide is in the form of a multimer.

36. – 40. (Canceled)

41. (Previously presented) The method of claim 29, wherein said composition is dispersed in a pharmaceutically acceptable aqueous medium.

42. (Previously presented) The method of claim 29, wherein said composition is administered at a dosage range of between about 10 micrograms to about 500 milligrams.

43. (Currently amended) The method of claim 40, wherein dosage range is about 50 micrograms to about 1 milligram.

44. (Previously presented) The method of claim 41, wherein said dosage range is about 100 micrograms.

45. (Previously presented) The method of claim 29, further comprising contacting said cell with said composition a second time.

46. (Canceled)

47. (Previously presented) The method of claim 29, wherein said contacting comprises injection of said composition.

48. (Canceled)

49. (Previously presented) A method for directly inhibiting HIV entry into a cell *in vitro* comprising the step of contacting said cell with a composition comprising a peptide of 8 to 24 residues comprising the sequence RAFVTIGK (SEQ ID NO:5).

IX. Evidence Appendix 2

Haynes *et al.*, US 5,019,387, cited in the Office Action dated 6/20/05

Koito et al, cited in the Office Action dated 6/20/05

Excerpt from Examiner's Answer dated 4/25/00, paper 21.

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
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Washington, D.C. 20231

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Paper No. 21

Application Number: 08/869,386
Filing Date: June. 5, 1997
Appellant(s): Sastry et al.

Stephen Hash
For Appellant

EXAMINER'S ANSWER

This is in response to appellant's brief on appeal filed Mar. 31, 2000.

(1) Real Party in Interest

A statement identifying the real party in interest is contained in the brief.

(2) Related Appeals and Interferences

A statement identifying the related appeals and interferences which will directly affect or be directly affected by or have a bearing on the decision in the pending appeal is contained in the brief.

(3) Status of Claims

The statement of the status of the claims contained in the brief is correct.

(4) Status of Amendments After Final

The appellant's statement of the status of amendments after final rejection contained in the brief is correct.

(5) Summary of Invention

The summary of invention contained in the brief is correct.

(6) Issues

The appellant's statement of the issues in the brief is substantially correct.

The issues are:

a. Claims 29-45 and 47 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of inhibiting HIV entry into a cell *in vitro* comprising contacting the cell with peptides consisting of SEQ ID NOs: 1, 3, or 5, does not reasonably provide enablement for a method of inhibiting HIV entry into a cell *in vivo* employing all of the possible claimed peptide sequences is maintained for reasons of record.

b. Claim 49 is rejected under 35 U.S.C. 102(e) as being anticipated by Berzofsky et al. (U.S. Pat. No. 5,820,865).

(7) Grouping of Claims

Claims 29-45 and 47 stand or fall together with regards to the 112 rejection. Claim 49 stands alone with regard to the 102 rejection.

(8) Claims Appealed

The copy of the appealed claims contained in the Appendix to the brief is correct.

(9) Prior Art of Record

The following is a listing of the prior art of record relied upon in the rejection of claims under appeal.

5,820,865	Berzofsky et al.	Oct. 13, 1998

(10) Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

a. Claims 29-45 and 47 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of inhibiting HIV entry into a cell *in vitro* comprising contacting the cell with peptides consisting of SEQ ID NOs: 1, 3, or 5, does not reasonably provide enablement for a method of inhibiting HIV entry into a cell *in vivo* employing all of the possible claimed peptide sequences is maintained for reasons of record.

The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims. The claims are drawn to a method of directly inhibiting HIV entry into a cell comprising contacting the cell with a composition comprising a peptide of 8-24

residues comprising SEQ ID NO:5. The specification at pages 65-67 and Fig. No. 8 disclose culturing MT-4 cells and primary human T cells in the presence of HIV and a selected peptide from the V3 loop of gp120 and the reverse transcriptase assays showed a decrease in the amount of reverse transcriptase produced in the cells incubated with certain peptides. However, the specification does not show a correlation between that which occurred *in vitro* to that which one of skill in the art would reasonably expect *in vivo*.

The specification provides no probative evidence to support the claimed treatment which would protect humans against HIV infection. The obstacles to treatment development and therapeutic approaches with regard to retroviruses associated with AIDS in humans are well documented in the literature. These obstacles include: 1) the extensive genomic diversity associated with the HIV retrovirus, particularly with respect to the gene encoding the envelope protein, 2) the fact that the modes of viral transmission include virus-infected mononuclear cells, which pass the infecting virus to other cells in a covert form, as well as via free virus transmission, 3) existence of a latent form of the virus, 4) the ability of the retrovirus to "hide" in the central nervous system where blood cells and neutralizing agents carried by the blood cannot reach the retrovirus, due to the blood-brain barrier and 5) the complexity and variation of the elaboration of the disease. The existence of these obstacles establish that the contemporary knowledge in the art would prevent one of ordinary skill in the art from accepting any vaccine or any immunization treatment or any therapeutic regimen on its face. In order to enable claims to drugs and their uses, either *in vivo* or *in vitro* data, or a

combination of these can be used. However, the data must be such as to convince one of ordinary skill in the art that the claims are sufficiently enabled. When the claims are directed to humans adequate animal data would be acceptable in those instances wherein one of ordinary skill in the art would accept the correlation to humans. Thus in order to rely on animal data there must exist an art-recognized animal model for testing purposes. See In re Hartop, 311 F.2d 249, 135 USPQ 419 (CCPA 1962).

Yarchoan et al. (J. Enz. Inh., 1992) state that while a number of agents have been found to block HIV binding to the target cell in vitro, these agents have generally not shown clear-cut evidence of clinical activity (abstract). Moreover, Gait et al. (TIBTECH 1995) discuss the problems associated with protein therapies for HIV and state that they suffer from problems of short serum half-life, poor bioavailability, and rapid clearance. Gait et al. also teach that as these problems were overcome, other problems emerged such as sequestration of the drug by serum proteins, drug resistance, and uneven distribution throughout the body, and that since these types of problems are unpredictable, it remains necessary to take into account the pharmacological parameters (p. 437).

Factors to be considered in determining whether a disclosure would require undue experimentation have been summarized in Ex parte Forman, 230 USPQ 546 (BPAI 1986)and reiterated by the Court of Appeals in In re Wands, 8 USPQ 2d 1400 at 1404 (CAFC 1988).

In the instant specification, it is determined that: 1) there are no working examples which suggest the desired results of inhibiting HIV infection *in vivo*, 2) the nature of the invention involved the complex and incompletely understood area of immunity to HIV, 3) the state of the prior art shows that prior treatment methods have been largely ineffective for the intended purpose, 4) the relative skill of those in the art is commonly recognized as quite high (post-doctoral level), and 5) the lack of predictability in the field to which the invention pertains is recognized in the art as evidenced by prior failures. In view of all of the above, it is determined that the specification is not commensurate in scope with the claimed invention.

b. Claim 49 is rejected under 35 U.S.C. 102(e) as being anticipated by Berzofsky et al. (U.S. Pat. No. 5,820,865). The claim is drawn to a method for directly inhibiting HIV entry into a cell *in vitro* comprising contacting the cell with a peptide comprising a specific sequence. It should be noted that the phrase "for directly inhibiting HIV entry into a cell" is viewed as an intended use and is given little patentable weight. Berzofsky et al. disclose a method for protecting cells from HIV comprising contacting cells *in vitro* with a composition that comprises a peptide having the claimed sequence (cols. 3-4). The method of Berzofsky et al. is the same as the claimed method. Therefore, Berzofsky et al. anticipate the invention as claimed.

(11) Response to Argument

a. Regarding the rejection of claims 29-45 and 47 under 35 U.S.C. 112, first paragraph, appellant urges that *in vitro* and *in vivo* data have been presented which enable the claimed invention, the *in vivo* data presented in the Arlinghaus declaration employs an accepted animal model, and Fultz, 1993 teaches that the chimpanzee is an acceptable model for HIV in humans.

This is not found persuasive because, as previously stated, Haynes (Science 1993) states that "in spite of an extraordinary amount of work in search of an animal model for human AIDS , no animal model exactly mirrors HIV infection". Haynes also states that the immune correlates of animal models to human regarding AIDS are not known (p. 1280 1st. col. 1st and 2nd. para.s) . Haynes, et al. (Ann. Med., 1996 p. 40) teach that major scientific obstacles blocking the development of successful HIV treatments are the extraordinary variability of HIV, the lack of an exact animal model of HIV-induced AIDS, and the lack of understanding of the correlates of protective immunity to HIV (p 1280). Regarding the use of *in vitro* data, Ex parte Balzarini, 21 USPQ 2d 1892 (BPAI, 1991) states that persons skilled in the art would question claims for *in vivo* treatment of retroviral diseases, AIDS, or AIDS-related diseases based upon *in vitro* testing, since references show that those skilled in art would not associate successful *in vitro* results with successful *in vivo* treatment of AIDS. It is clear that one of skill in the art at the time of applicant's invention would not have expected *in vitro* data or *in vivo* data employing chimpanzees to correlate to humans. While Fultz et al. discusses

using chimpanzees as a model for screening potential HIV treatments and that chimpanzees can become infected with HIV, Haynes et al., 1996 (which was published three years after Fultz et al.) states that current animal models of either HIV or simian (such as the chimpanzee) fall short of precisely mirroring HIV infection in humans (p. 41, 2nd complete par.). Additionally, Gait et al. (TIBTECH 1995, published two years after Fultz et al.) discuss the problems associated treating HIV infection in humans with peptide therapies and state that peptide therapies suffer from problems of short serum half-life, poor bioavailability, and rapid clearance. Gait et al. also teach that as these problems were overcome, other problems emerged such as sequestration of the drug by serum proteins, drug resistance, and uneven distribution throughout the body, and that since these types of problems are unpredictable, it remains necessary to take into account the pharmacological parameters (p. 437). Therefore, it is clear that one of skill in the art at the time the invention was made would not have expected *in vitro* or *in vivo* data to correlate to directly inhibiting HIV infection in human cells. Moreover, the claims recite a method of protecting human cell from HIV infection which reads on a vaccine for HIV. Regarding the state of HIV vaccine as of 1993, well after filing date of the present invention, Wright states that because of the high degree of genetic, antigenic variations in such viruses, no one has yet, years after the invention, developed a generally successful AIDS virus vaccine. Wright 999 F. 2d at 1561, 27 USPQ2d at 1513.

Appellant further urges peptides are an accepted means of inhibiting viral uptake and cites Vitetta et al. and Sabatier et al. and a closely related peptide is now in phase II clinical trials.

This is not found persuasive because Yarchoan et al. (J. Enz. Inh., 1992) state that while a number of agents have been found to block HIV binding to the target cell in vitro, these agents have generally not shown clear-cut evidence of clinical activity (abstract). Moreover, Gait et al. (TIBTECH 1995) discuss the problems associated with peptide therapies for HIV and state that they suffer from problems of short serum half-life, poor bioavailability, and rapid clearance. Gait et al. also teach that as these problems were overcome, other problems emerged such as sequestration of the drug by serum proteins, drug resistance, and uneven distribution throughout the body, and that since these types of problems are unpredictable, it remains necessary to take into account the pharmacological parameters (p. 437). Additionally, it is well known, as shown by the attached FDA 257a study that Phase II studies are concerned with safety and not treatment of disease. It should be noted that the two patents cited by appellant do not claim a method of directly inhibiting HIV infection into a human cell and are not within the scope of the claimed invention.

Appellant further urges that the Arlinghaus declaration shows inhibition of HIV replication which denotes an inhibition of cellular infection rather than prevention of host infection.

This is not found persuasive because the Arlinghaus declaration states that the peptides inhibit HIV replication *in vitro* or in chimpanzees which is not the same as directly inhibiting HIV infection. Therefore, the declaration is not commensurate in scope with the claimed invention.

Furthermore, Appellant urges the working examples in the specification and the teachings of the specification enable the invention as claimed.

This is not found persuasive because, as stated above, Haynes (Science 1993) states that "in spite of an extraordinary amount of work in search of an animal model for human AIDS , no animal model exactly mirrors HIV infection". Haynes also states that the immune correlates of animal models to human regarding AIDS are not known (p. 1280 1st. col. 1st and 2nd. para.s) . Haynes, et al. (Ann. Med., 1996 p. 40) teach that major scientific obstacles blocking the development of successful HIV treatments are the extraordinary variability of HIV, the lack of an exact animal model of HIV-induced AIDS, and the lack of understanding of the correlates of protective immunity to HIV (p 1280). Regarding the use of *in vitro* data, Ex parte Balzarini, 21 USPQ 2d 1892 (BPAI, 1991) states that persons skilled in the art would question claims for *in vivo* treatment of retroviral diseases, AIDS, or AIDS-related diseases based upon *in vitro* testing, since references show that those skilled in art would not associate successful *in vitro* results with successful *in vivo* treatment of AIDS. It is clear that one of skill in the art at the time of applicant's invention would not have expected *in vitro* data or *in vivo* data employing chimpanzees to correlate to humans.

b. Regarding the rejection of claim 49 is rejected under 35 U.S.C. 102(e) as being anticipated by Berzofsky et al., appellant urges that the mechanisms of protection sought by the claimed invention and the Berzofsky et al. patent are different, Berzofsky et al. fail to teach expressly or inherently each limitation of the claimed invention and one of ordinary skill in the art would not extrapolate from the reference that the method be used for directly inhibiting viral entry.

This is not found persuasive because the claim is drawn to a method for directly inhibiting HIV entry into a cell *in vitro* comprising contacting the cell with a peptide comprising a specific sequence. The phrase "for directly inhibiting HIV entry into a cell" is viewed as an intended use and is given little patentable weight. In response to applicant's arguments, the recitation "for directly inhibiting HIV entry into a cell" has not been given patentable weight because the recitation occurs in the preamble. A preamble is generally not accorded any patentable weight where it merely recites the purpose of a process or the intended use of a structure, and where the body of the claim does not depend on the preamble for completeness but, instead, the process steps or structural limitations are able to stand alone. In re Hirao, 535 F.2d 67, 190 USPQ 15 (CCPA 1976); Kropa v. Robie , 88 USPQ 478, 481

(CCPA 1951). As previously stated, Berzofsky et al. disclose a method for protecting cells from HIV comprising contacting cells *in vitro* with a composition that comprises a peptide having the claimed sequence (cols. 3-4). The claims are broadly written and do not recite the mechanism for inhibiting infection of the cell. Therefore, since the method of Berzofsky et al. employs the same active ingredient and the same step,it is the same as the claimed method. In conclusion, Berzofsky et al. anticipate the invention as claimed.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

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X. Related Appeals and Interferences Appendix 3

None

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,019,387

DATED : May 28, 1991

INVENTOR(S) : Haynes et al

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 2, line 56, correct the sequence to read: --CTRPNNNTRKSIRIQRGPG--.

Column 13, claim 3, line 3, correct the sequence to read: --CTRPNNNTRKSIRIQRGPG--.

Column 14, claim 11, line 3, correct the sequence to read: --CTRPNNNTRKSIRIQRGPG--.

Signed and Sealed this

Thirty-first Day of August, 1993

Attest:

BRUCE LEHMAN

Attesting Officer

Commissioner of Patents and Trademarks

International Immunology, Vol. 1, No. 6

A neutralizing epitope of human immunodeficiency virus type 1 has homologous amino acid sequences with the active site of inter-α-trypsin inhibitor

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Key words: epitope β, HIV, inter-α-trypsin inhibitor

Abstract

A neutralizing epitope (epitope β) of the HTLV-IIIB strain of HIV-1 was mapped to 24 amino acids of an external envelope glycoprotein (gp120) using a neutralizing monoclonal antibody (0.5β) and hetero-antisera against synthetic peptides encoding gp120. Proteins that have homologous sequences with epitope β were sought from a databank of protein sequences to assess biological features of epitope β. The results showed that epitope β was found to have homologous sequences to inter-α-trypsin inhibitor (ITI). The homologous region of ITI included the active site of the protein. Synthesized peptides including epitope β were good substrates for trypsin, because these peptides inhibited trypsin activities in a competitive manner (K_i = 24.5 μM). Human urinary trypsin inhibitor (UTI), a protein indistinguishable from ITI, as well as synthetic peptides including epitope β inhibited syncytium formation caused by the LAV-1-infected CCRF-CEM and uninfected Molt-4 cells in a dose-dependent manner (0.1 – 1 mM). These findings suggest that epitope β of HIV-1 could be substrate of protease upon HIV-1 infection and also suggest that protease inhibitory activity of epitope β may play a role in the pathophysiology of HIV-1-infected individuals.

Introduction

HIV-1 is etiologically associated with AIDS (1 – 3). The initial event of HIV-1 infection is assumed to be an attachment of a mature envelope glycoprotein (gp120) of HIV-1 to its cellular receptor, a CD4 molecule, present on the surface of helper T lymphocytes, macrophages, and other kinds of cell (4,5).

The exact mechanisms for the entry of the HIV-1 nucleocapsid into susceptible cells after binding to CD4 molecules have not been clarified yet, but this internalization seems to occur rapidly via a direct fusion of the virus envelope with the plasma membrane of the target cells. Recently, it has been clarified that receptor-mediated endocytosis is not required *in vitro* (6 – 8). To understand the mechanisms of this process, we previously raised a murine monoclonal antibody (mAb) (0.5β) against gp120 that specifically prevented the infection of HTLV-IIIB and LAV-1 strains of HIV-1. It was also revealed that 0.5β recognized 24 amino acids, 308 – 331 of gp120 of HIV-1 (9), and that this epitope is located at different sites of the CD4 binding portion of the envelope glycoprotein of HIV-1, which are believed to be located at residues 420 – 463 (10,11). We shall call the neutralizing epitope sequences epitope β hereafter. This region resides within a hypervariable region; however, there is a conserved sequence, Gly-Pro-Gly-Arg (GPGR), allowing a β-turn in the middle of epitope β (12). A hetero-antiserum which was able to neutralize the HAT-3 strain of HIV-1 was also successfully produced by immunizing synthetic peptides corresponding to the epitope β site of the HAT-3 strain in goat (13). Therefore epitope β has been thought to be an essential domain of gp120 in HIV-1 infection. In the present study we attempted to clarify biochemical features of this epitope.

Methods

Local sequence homology search

The study was carried out using the IDEAS system (Institute for Chemical Research, Kyoto University); the search was performed

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against the National Biomedical Research Foundation (NBRF) database. The measurement of similarity was based on amino acid mutation data complied as previously described (14). The deletion was set to eight.

Synthetic peptides

HIV-1 *env*- and *gag*-encoded peptides were synthesized according to the amino acid sequences of the BH-10 clone (15). Peptides were synthesized using a peptide synthesizer (Applied Biosystems 430A, Foster City, CA, USA) with chemicals and program cycles supplied by the manufacturer. Three peptides corresponding to gp120 were used in this study: CTR36 (Cys-Thr-Arg-Pro-Asn-Asn-Asn-Thr-Arg-Lys-Ser-Ile-Arg-Gln-Arg-Gly-Pro-Gly-Arg-Ala-Phe-Val-Thr-Ile-Gly-Lys-Ile-Gly-Asn-Met-Arg-Gln-Ala-Gln-Ala-His-Cys; 303 – 338), NNT-24 (308 – 331), and GPGR (318 – 321). The following control peptides were arbitrarily chosen from hydrophilic sequences of *gag*-encoded amino acids and were synthesized: TLL15 (Thr-Leu-Leu-Val-Gln-Asn-Ala-Asn-Pro-Asp-Cys-Lys-Thr-Ile-Leu; 320 – 334), ETI15 (Glu-Thr-Ile-Asn-Glu-Glu-Ala-Ala-Glu-Trp-Asp-Arg-Val-His-Pro; 203 – 217), GQM15 (Gly-Gln-Met-Arg-Glu-Pro-Arg-Gly-Ser-Asp-Ile-Ala-Gly-Thr-Thr; 226 – 240), and FYK21 (Phe-Tyr-Lys-Thr-Leu-Arg-Ala-Glu-Gln-Ala-Ser-Gln-Glu-Val-Lys-Asp-Trp-Met-Thr-Met-Glu-Thr; 300 – 320). After synthesis, the peptides were deprotected and cleaved from the supporting resin with trifluoromethanesulfonic acid. The peptide products were precipitated with ethylether and collected by filtration. After resolubilization in the minimum quantity of trifluoroacetic acid required and in sufficient amounts of 50% (v/v) acetonitrile, these peptides were lyophilized, reconstituted with 5% (v/v) acetic acid, and desalted on a Sephadex G25 column equilibrated with the same acetic acid solution. The desalted peptides were purified by reverse-phase HPLC (YMCD-ODS-5 column; 5 – 60% acetonitrile gradient in 0.1% trifluoroacetic acid in water).

ELISA

Synthetic peptides (CTR36, NNT24, GPGR) were coated to microtiter plates (Immulon 2, Dynatech Laboratories) and reactivities against 0.5β were measured as described previously (16).

Enzymatic assay for trypsin

The enzymatic activity of trypsin was measured with Boc-Phe-Ser-Arg-MCA (Peptide Institute, Inc., Osaka, Japan) as the substrate as described elsewhere (17). Ten microliters of TPCK-trypsin (final concentration 100 pg/ml, Sigma type XIII) were pre-incubated for 3 min at 37°C with 20 μl of the synthetic peptides in 950 μl of a reaction buffer (50 mM Tris – HCl, pH 8.0, containing 150 mM NaCl, 1 mM $CaCl_2$, and 1 mg/ml BSA). The amidolytic assay was then performed by the addition of 20 μl of the substrate, and the amounts of AMC released from the substrate were continuously monitored by a fluorescence spectrophotometer (Shimadzu RF 5000, Shimadzu Co., Kyoto, Japan) measuring absorbance at 380 nm and emission at 440 nm. The degree of peptide inhibitory effects was expressed as relative enzymatic activity compared to that of trypsin with the vehicle buffer. The mode of inhibition by the peptides was analyzed using a Lineweaver – Burk plot in the absence or presence of 20 μM of each peptide, and Dixon plots in the presence of either 100 or 25 μM of the substrate.

HIV-1-induced syncytia inhibition assay

HIV-1 (LAV-1)-infected CCRF-CEM cells (2×10^4 cells/well) and Molt-4 clone 8 cells (1×10^5 cells/well), which are highly susceptible to HIV-1 infection (18), were washed twice with RPMI 1640 (Nissui, Tokyo, Japan) and co-cultured in 96-well microtiter plates (Falcon, Oxnard, CA, USA) with serially diluted protease inhibitors and synthetic peptides. The co-culture was done in serum-free medium, ASF-104 (Ajinomoto, Tokyo, Japan), in a humidified atmosphere with 5% CO_2 at 37°C and the plates were intermittently shaken. After 12 h of culture, syncytia formation was examined under an inverted microscope (magnification ×200). The number of the multinucleated giant cells in each well was counted in duplicate determinations. Urinary trypsin inhibitor (UTI) was purified from the urine of pregnant women as previously described (19) and kindly provided by Japan Chemical Research Pharmaceutical Co. Ltd (Kobe, Japan). Soybean trypsin inhibitor (SBTI, 109 886) was from Boehringer-Mannheim Laboratories (München, FRG) and *p*-amidinophenylmethanesulfonylfluoride hydrochloride (p-APMSF, 014-10391) was from Wako Pure Chemical Industries Ltd (Kyoto, Japan). Aprotinin (A 4529), Leupeptin (L 2884), and Pepstatin (P 4265) were from Sigma (St Louis, MO, USA).

Cell proliferation assay

Because protease inhibitor may inhibit various cellular metabolisms, the effects of these inhibitors on [^{3}H]thymidine ([^{3}H]TdR) uptake by cells were examined. One hundred thousand Molt-4 clone 8 cells and 20,000 CCRF-CEM cells in 150 μl of medium (ASF 104) were cultured for 36 h at 37°C with serially diluted UTI in flat-bottomed plates (Falcon Microtest Plates III). The cultured cells in triplicate wells were pulsed with [^{3}H]TdR (Amersham, Tokyo, Japan) (0.5 μCi/well) during the final 8 h of the culture. After the separation of cells from free [^{3}H]TdR with glass fiber filters, the radioactivity incorporated into the cells was counted by liquid scintillation.

Immunoprecipitation and gel electrophoretic analysis of radio-labeled antigen

HIV-1 (LAV-1)-infected U937 cells and non-infected U937 cells were washed three times with Hank's balanced salt solution (HBSS) and cultured in cysteine-free RPMI 1640 medium (Nissui, Tokyo, Japan) supplemented with 20% dialyzed fetal calf serum (FCS; GIBCO, Grand Island, NY, USA) at a concentration of 100 μCi/ml of [^{35}S]cysteine (Amersham, Tokyo). After cultivation for 6 h, these labeled cells were washed with RPMI 1640 medium and co-cultured with unlabeled Molt-4 clone 8 or HL-60 cells for another 3, 6, and 12 h. The cell aggregates or syncytia formed in the co-culture were washed with RPMI 1640 medium three times, homogenized, and the membrane proteins were extracted. These labeled antigens in the extract were immuno-precipitated with 0.5β mAb as well as with the serum of an HIV-1-infected subject, and were analyzed by 12% SDS – PAGE, followed by autoradiography (20).

Results

Proteins bearing amino acid sequences homologous with epitope β

A search against the latest NBRF database showed that 148 proteins have homologous sequences with epitope β.

Interestingly, 43 proteins are derived from various viruses including gp120 of four different HIV-1 strains, but we could not find proteins of other viruses which play a role in infection. Eighteen proteins are from human, and inter-α-trypsin inhibitor gave the highest homology score and percentage match (−42 and 46.7% respectively). In addition, the homologous region included the conserved sequences of epitope β (GPGR; Fig. 1). Three proteins are protease and five proteins are protease inhibitors; ITI is the only human protein. ITI from other species were also found and gave the highest scores. Homologous regions of ITI reside in the active sites of the inhibitor (21). These coincidental findings suggest that epitope β might have similar biochemical activities to ITI and the similarities might be biologically significant. Thus, we have synthesized two peptides (CTR36, NNT24) including epitope β and other control peptides.

Effects of synthetic peptides on enzyme activities

Using ELISA, it was confirmed that 0.5β reacted with CTR36 and NNT24 but not with GPGR peptide, and that the control mAb (MOPC 21) did not recognize them (Fig. 2). The epitope β-like peptides apparently inhibited the amidolytic activity of trypsin on the substrate Boc-Phe-Ser-Arg-MCA, although negligible or weak inhibitory effects on trypsin activities by the control peptides (TLL15, ETI15, GQM15, FYX21) were observed (Fig. 3A). The mode of inhibition by the peptides on trypsin activities was examined. In the presence of either CTR36 or NNT24, the K_m value of Boc-Phe-Ser-Arg-MCA on trypsin activities decreased, while V_{max} value calculated from the Lineweaver – Burk plots did not change (Fig. 3B-a). The evaluated K_i values using Dixon plots of CTR36 and NNT24 were 12.5 and 24.2 μM respectively (Fig. 3B-b and -c). These results indicated that the mode of inhibition caused by these peptides was competitive.

(Protein)	(Number)	
BH-10	306	S I R I Q R G P G R A F V T I
HAT-3	308	S I T K − − G P G R V I Y A T
ITI (pig)	62	S L P I V Q G P C R A F I R L
ITI (bovine)	62	N L P I V Q G P C R A F I Q L
ITI (horse)	62	N L P I V Q G P C R A F I R L
ITI (human)	62	N L P V I R G P C R A F I Q L

Fig. 1. Amino acid alignment of BH-10 and HAT-3 in the regions corresponding to the neutralizing epitope with inter-α-trypsin inhibitor (ITI). A one-letter amino acid code is used and the box indicates homologous regions.

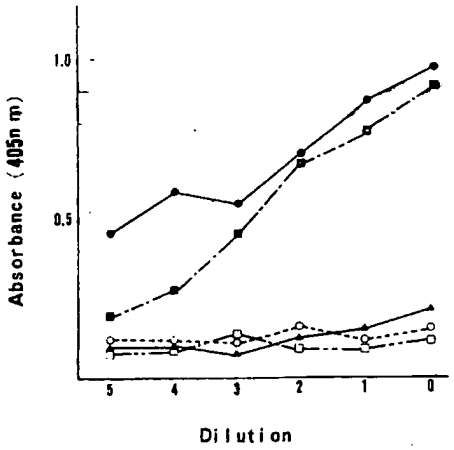

Fig. 2. Binding of 0.5β with synthetic peptides in ELISA. Synthetic peptides (CTR36, NNT24, GPGR) were purified using HPLC and coated to microtiter plates with 1 μg/ml of each peptide. Purified IgG (3 μg/ml) of 0.5β after serial dilution (1:3) was added to the plates which were coated with CTR36 (●), NNT24 (■), and GPGR (▲). Control IgG (MOPC 21) was also added to CTR36 (○) and NNT24 (□) coated plates.

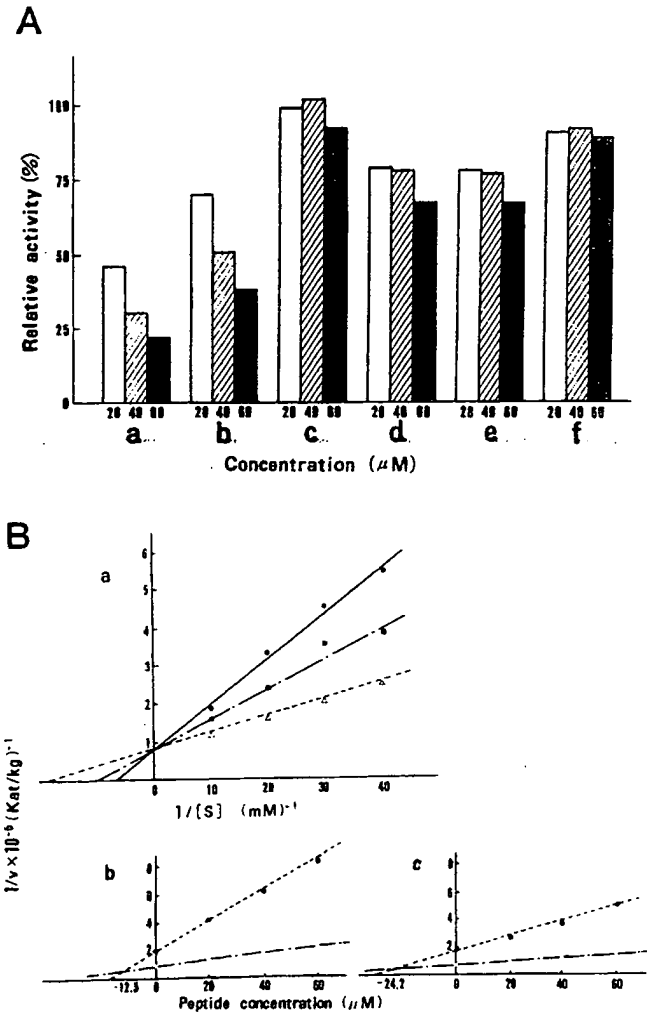

Fig. 3. Inhibition of trypsin activity by synthesized peptides including epitope β. Synthesized peptides were incubated with TPCK-trypsin and the substrate (Boc-Phe-Ser-Arg-MCA), then the relative trypsin activities of the samples at various concentrations of peptides were examined. CTR36 and NNT24 were from *env* gp120, and TLL15, ETI15, GQM15, and FYK21 were from amino acid residues of *gag* p24 respectively. (A) a, CTR36; b, NNT24; c, TLL15; d, ETI15; e, GQM15; f, FYK21. (B) The inhibition pattern of synthesized peptides on trypsin. (a) Effect of substrate concentration on the trypsin activity in the presence (●, 20 μM CTR36; ■, 20 μM NNT24) or absence (△) of peptides using a Lineweaver – Burk plot. Effect of substrate concentration (●, 0.025 mM; ○, 0.1 mM of Boc-Phe-Ser-Arg-MCA) on the trypsin hydrolysis using a Dixon plot; (b) CTR36; (c) NNT24. All experiments were done in duplicate.

Interference of HIV-1 envelope-induced syncytium formation by trypsin inhibitors and epitope β-like peptides

In order to elucidate if some protease(s) may play an important role in syncytium formation induced by HIV-1, the effects of various inhibitors were examined. UTI inhibited syncytium formation in a dose-dependent manner. No remarkable effects on cell viabilities or growth rates were observed at the concentration of UTI used in these experiments (Fig. 4). The effects of various protease inhibitors on syncytium formation are summarized in Table 1. PMSF, aprotinin, leupeptin, and pepstatin showed no inhibitory effects on syncytium formation at the concentrations examined. Soybean trypsin inhibitor (SBTI) inhibited syncytium formation in a dose-dependent manner (0.3 – 1 mM) (Table 1). In contrast, apparent inhibition was achieved at a 10-fold lower concentration by UTI (0.03 – 1 mM). To clarify whether inhibitory activities by UTI are specific or not, the numbers of giant cells formed and the effects on [^{3}H]TdR uptake of UTI were examined simultaneously. A 50% decrease in giant cell formation was observed at 100 μM, but no effect on [^{3}H]TdR uptake was seen at this concentration. But a slight decrease in [^{3}H]TdR uptake was observed at 1 mM of UTI. These findings ruled out the possibility of non-specific inhibition by this inhibitor (Fig. 4). Strong inhibition of the syncytium formation was observed when synthetic peptides (CTR36, NNT24) were added to the co-cultivation (Table 1). These inhibitory effects of synthetic peptides at a concentration of 300 μM were not seen by absorbing with an optimal amount (30 μg/ml) of 0.5β (data not shown).

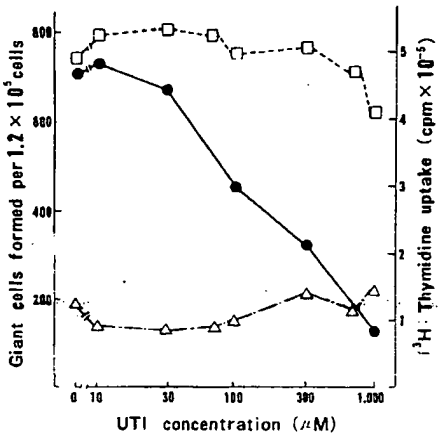

Fig. 4. Effects of UTI on syncytium formation (giant cells) and on cell proliferation. The numbers of giant cells were assessed. ● represents the means of duplicate determinations. Proliferation of Molt-4 clone 8 cells (□, 1 × 10^5 cells/well) and CCRF-CEM cells (△ 2 × 10^4 cells/well) were examined in triplicate at different UTI concentrations.

Table 1. Effects of protease inhibitors and synthesized peptides on syncytium formation

Reagents	Final concentration of reagents					
	3 mM	1 mM	300 μM	100 μM	30 μM	10 μM
Protease inhibitors						
UTI	NT[a]	++[b]	+[c]	+	±[d]	−[e]
SBTI	++	+	±	−	−	−
PMSF	−	−	−	−	−	−
Aprotinin	−	−	−	−	−	−
Leupeptin	−	−	−	−	−	−
Pepstatin	−	−	−	−	−	−
Synthetic peptides						
CTR36	NT	++	+	+	±	−
NNT24	NT	++	+	±	−	−
GPGR	−	−	−	−	−	−
TLL15	NT	−	−	−	−	−
ETI15	NT	−	−	−	−	−

[a]NT, not tested; [b]++, >60% inhibition; [c]+, 30 – 60% inhibition; [d]± 10 – 30% inhibition; [e]−, no inhibition.

Radio-immunoprecipitation of gp120 of HIV-1 during syncytium formation

The above findings suggested that epitope β may interact with protease and that epitope β might be a cleavage site of gp120 for successful infection of HIV-1. If this is the case, degradation of gp120 would be detected during syncytium formation. Thus, we performed a radio-immunoprecipitation assay, before and during syncytium formation. LAV-1-infected U937 cells were radiolabeled and cultured with Molt-4 clone 8 for several hours, and cellular extracts were immunoprecipitated as described in Methods. However, HIV-1 envelope cleavage products with small molecular weights were not clearly detected using 0.5β and the HIV-1-infected serum (data not shown).

Discussion

The data reported here demonstrate that synthetic peptides, corresponding to the neutralizing epitope of HIV-1 gp120 (epitope β), has a region that is significantly homologous with the active site of ITI. The epitope has also been shown to be biochemically active, since epitope β had an inhibitory activity on trypsin in a competitive manner. Inhibition by CTR36 was apparently more potent than that by NNT24. It is already known that the neutralizing epitope forms a loop between the two cross-linked cysteine residues (12). Thus, it is possible that cysteines from both sides of CTR36 may play a role in forming three-dimensional structures of the peptides, thereby acting as a more potent inhibitor. The synthetic peptides, as well as UTI, inhibited syncytium formation, probably because they inhibited cellular protease(s) necessary for HIV infections, as discussed below. It was reported that there is no inhibitory activity of the recombinant gp120 fragment including this neutralizing epitope at 4 μM (22). Higher concentrations, ranging from 30 μM to 1 mM of the peptides used in the present experiment, may explain the different results. CTR36 was more active than NNT24, confirming the results obtained by an enzyme assay and suggesting that the biochemical and biological activities were closely associated. The biological significances of competitive inhibition of protease by the neutralizing epitope of HIV-1 are not clear, but several possibilities exist.

Firstly, the epitope may be cleaved by cellular protease, because synthetic peptides corresponding to epitope β were good substrates of trypsin. It has already been suggested that endoproteolytic cleavage of HIV-1 envelope polyprotein (gp160) to a gp120-gp41 heterodimer and further proteolysis of gp120 by cellular protease(s) are necessary for subsequent infection (23). It is not clear whether either UTI or ITI might be beneficial

for a therapeutical approach to HIV-infected individuals, because ITI is present in the serum and the concentrations of ITI in the sera of normal individuals is ~2.8 μM. The high concentration (0.03 – 1 mM) needed to inhibit syncytium formation described here suggests that the usage might not be practical. Recently, we have reported that the cellular Kunitz-type protease inhibitor from rat mast cells, trypstatin, which also has a homologous sequence with ITI, inhibited syncytium formation at a far lower concentration (1 μM). Furthermore, anti-tryptase antibody inhibited syncytium formation, suggesting involvement of tryptase-like enzymes in HIV-1 infection (24). Although radio-immunoprecipitation followed by autoradiography did not show any breakdown products during syncytium formation, our experiments raise the possibility that epitope β is a target substrate for cellular protease which may cleave gp120 upon infection and facilitate subsequent membrane fusion. Taken together, it is necessary to characterize the mode of involvement of protease(s) in HIV infection using HIV-sensitive human cell lines.

Secondly, the observations reported here might explain one of the potential roles of neutralizing epitope in the pathogenesis of HIV-1 infection, because ITI as well as gp120 was reported to have immunosuppresive effects *in vitro* (25,26). Thus, it is possible that the synthetic peptides corresponding to epitope β might have immunomodulating activities, and these studies are now underway. It is also of note that ITI has homologous sequences with amyloid precursor proteins, which are believed to be etiologically associated with Alzheimer's dementia (27), and the presence of Alzheimer's type II astrocytes was also reported in patients with AIDS-dementia complex (28).

In conclusion, further studies of the biological significance of the neutralizing epitope of HIV-1 are needed to analyze the pathophysiology of HIV-1 infection as well as therapeutical approaches in HIV-1-infected individuals.

Acknowledgements

We are grateful to Dr L. Montagnier for supplying LAV-1 and Dr N. Yamamoto for the Molt-4 clone 8 cell line. We also thank Drs H. Iwamoto, M. Kato, H. Kido, T. Shoji, and J. Tanaka for their helpful discussions. This work was supported by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science, and Culture of Japan.

Abbreviations

ITI	inter-α-trypsin inhibitor
NBRF	National Biomedical Research Foundation
UTI	urinary trypsin inhibitor

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